```
=> d his

     (FILE 'HOME' ENTERED AT 20:42:37 ON 24 JUL 2007)
     FILE 'CA' ENTERED AT 20:42:54 ON 24 JUL 2007
L1  317116 S PHOSPHORYLAT? OR GLYCOSYLAT? OR DEACYLAT? OR ACYLAT?
L2  507353 S PHOSPHOKINASE OR KINASE OR TRANSFERASE OR PHOSPHORYLASE OR
           TRANSAMINASE OR AMINASE OR CARBOXYLASE OR CARBONYLASE OR
           PHOSPHATASE
L3  111473 S L1 AND L2
L4    3190 S L3 AND (FLUORESC? OR FLUORIMET? OR FLUOROMET?)
L5    3834 S L3 AND PEPTIDE AND SUBSTRATE
L6     255 S L4 AND L5
L7      61 S L6 AND PY<2000
L8   29250 S L3 AND (PEPTIDE OR OLIGOPEPTIDE OR SUBSTRATE)
L9    1035 S L4 AND L8
L10     87 S L9 AND QUENCH?
L11    319 S L9 AND PY<2000
L12    206 S L11 AND(HOMOGENEOUS OR ASSAY? OR DETERMIN? OR MICRODETERMIN? OR
           MEASUR? OR MONITOR? OR SENSE# OR SENSOR OR SENSING OR DETECT?)
L13    287 S L7,L10,L12
     FILE 'BIOSIS' ENTERED AT 21:10:37 ON 24 JUL 2007
L14    213 S L13
     FILE 'MEDLINE' ENTERED AT 21:11:11 ON 24 JUL 2007
L15    392 S L13
     FILE 'BIOSIS' ENTERED AT 21:13:12 ON 24 JUL 2007
L16    196 S L14 AND PY<2000
     FILE 'MEDLINE' ENTERED AT 21:13:44 ON 24 JUL 2007
L17    369 S L15 AND PY<2000
     FILE 'BIOSIS' ENTERED AT 21:17:15 ON 24 JUL 2007
L18     85 S L7,L10
L19     68 S L18 AND PY<2000
     FILE 'MEDLINE' ENTERED AT 21:18:33 ON 24 JUL 2007
L20    165 S L7,L10
L21    142 S L20 AND PY<2000
L22      8 S L17 NOT L21 AND(FLUORESC? OR FLUORIMET? OR FLUOROMET?)/TI
     FILE 'CA, BIOSIS, MEDLINE' ENTERED AT 21:24:29 ON 24 JUL 2007
L23    369 DUP REM L13 L19 L21 L22 (136 DUPLICATES REMOVED)

=> d bib,ab l23 1-369

L23  ANSWER 51 OF 369  CA  COPYRIGHT 2007 ACS on STN
AN   138:35759  CA
TI   Fluorescent protein sensors containing phosphorylation sites introduced
     by N-terminal mutagenesis
IN   Cubitt, Andrew B.
PA   Aurora Biosciences Corporation, USA
SO   U.S., 49 pp.
PI   US 6495664          B1    20021217    US 1998-129192        19980724
PRAI US 1998-129192      A1    19980724
AB   The present invention includes a fluorescent compd. that can detect an
     activity, such as an enzymic activity, and exhibits quenching.  The
     fluorescent compd. is a fluorescent protein, such as an Aequorea-related
     green fluorescent protein..  The green fluorescent protein (GFP) of
```

Aequorea victoria is modified to include a **substrate** site for an enzymic activity such as a **kinase** activity, a **phosphatase** activity, a protease activity, and a glycosylase activity. Thus, relative **fluorescence** of **phosphorylated** vs. non-**phosphorylated** GFP is enhanced by modifying the N-terminal region (e.g., residues MSKGEELF to MGRRRASII) to contain a **phosphorylation** site responsive to protein **kinase** A, or other protein **kinase** enzymes,. Addnl. amino acid substitutions are engineered (S65A, K79R, E90N, N149K, V163A, I167T, and optionally A87T and E90A) to further improved **fluorescence** yield. The **fluorescent** compd. of the present invention can be used to detect such enzymic activities in samples, such as biol. samples, including cells. The present invention also includes nucleic acids that encode the **fluorescent** compds. of the present inventions, and cells that include such nucleic acids or **fluorescent** compds.

L23 ANSWER 53 OF 369 CA COPYRIGHT 2007 ACS on STN
AN 134:128210 CA
TI Homogeneous **fluorescence** method for assaying structural modifications of biomolecules using double-labeled **substrates**
IN Blumenthal, Donald K., II
PA University of Utah Research Foundation, USA
SO PCT Int. Appl., 35 pp.
PI WO 2001007638 A2 20010201 WO 2000-US40495 20000727
PRAI US 1999-145755P P 19990727
AB Double-labeled protein biomol. **substrates** and methods for the homogeneous assay of processes by which biomols. are covalently modified are described. The methods of the present invention utilize biomol. **substrates** labeled at two positions with two **fluorescent** dyes or with a **fluorescent** dye and a nonfluorescent dye. The two labeling dyes of the unmodified biomol. **substrates** stack, thereby **quenching** the **substrate**'s **fluorescence**. Upon covalent modification of the double-labeled **substrate**, however, the intramolecularly stacked dyes dissoc. and the **fluorescence** of the **phosphorylated substrate** changes markedly. Methods utilizing the double-labeled **substrates** of the present invention do not require phys. sepn. of modified and unmodified **substrate** mols., nor do they require other special reagents or radioactive materials. Methods for prepg. and characterizing the **substrates** used in the assay procedure are described, as are methods utilizing the **substrates** of the present invention for high-throughput screening, for monitoring intracellular processes of covalent biomol. modification in living cells, for diagnostic and therapeutic applications for diseases involving dysfunctional processes of covalent biomol. modification, and for discovering novel enzymic **substrates**. A synthetic KID **peptide** was prepd. and double-labeled with tetramethylrhodamine-5-maleimide and 5-carboxyfluorescein, succinimidyl ester or 5-carboxytetramethylrhodamine, succinimidyl ester. These **substrates** can be used to assay for protein **kinase** A as the **phosphorylated substrates** have detectable changes in the absorbance and **fluorescence** characteristics of the dyes included in the **substrates**.

L23 ANSWER 69 OF 369 CA COPYRIGHT 2007 ACS on STN
AN 130:153976 CA

TI Preparation of **peptides** having **fluorescence** and **fluorescence-quenching** groups as **substrates** for **determination** of protein **phosphatase**
IN Nishikata, Makoto
PA Japan
SO Jpn. Kokai Tokkyo Koho, 11 pp.

PI	**JP 11012297**	**A**	**19990119**	**JP 1998-126684**	**19980421**
	US 5917012	**A**	**19990629**	**US 1998-70756**	**19980430**
PRAI	JP 1997-126463	A	19970430		

AB (un)protected **peptides** represented by formula A1-X-A3 [X = PO3H2-introduced amino acid residue; A1, A2 = (un)protected amino acid or **peptide** residue linked to X; either of A1 or A2 possesses a **fluorescence-quenching** group at the terminus or side-chain and the other possesses a **fluorescent** group possessing **fluorescence** property by its self at the terminus or side-chain with its **fluorescence** property being **quenched** by a **fluorescence-quenching** group in the mol.] are prepd. A method for **detn.** of protein **phosphatase** comprises reaction of the above **peptide** deriv. with a protein **phosphatase**, reaction of the reaction product with protease, and **measurement** of the change in **fluorescence** intensity. A reagent for **detn.** of protease **phosphatase** activity contains the above **peptide** deriv. and protease which selectively cleaves the **peptide** bond between the **fluorescence-quenching** group-contg. amino acid residue and the **fluorescence** group-linked amino acid residue after dephosphorylation from PO3H2-introduced amino acid residue by protease **phosphatase**. The advantages of these **peptide substrates** are (1) protein **phosphatase** activity is **measured** in high sensitivity, since the **assay measures fluorescence** intensity, (2) they can be stably stored for a long period of time, since they do not contain radio active phosphorus compds., (3) the **assay** can be applied to a sample contg. phosphate ions, since it does not **measure** released phosphate ions, (4) a crude tissue ext. liq. can be also used as a sample, since the excitation wavelength is set to the wavelength different from the absorption frequency of proteins by appropriately choosing the **fluorescence** group, and (5) dephosphorylation reaction of the **substrates** can be traced in real time as long as protein **phosphatase** to **detd.** is resistant to protease having the above property. Thus, Mca-Gly-Glu-Gly-Thr-Tyr(PO3H2)-Gly-Lys(DNP)-Arg-NH2 (DNP = 2,4-dinitrophenyl, Mca = 7-methoxycumarin-4-ylacetyl), which was prepd. by the solid phase method using Nα-Fmoc-protected amino acids and a MBHA resin, enabled **detn.** of protein tyrosine **phosphatase** in real time with good accuracy.

L23 ANSWER 94 OF 369 MEDLINE on STN
AN 1999441240 MEDLINE
DN PubMed ID: 10510304
TI A phosphotyrosine-containing **quenched** fluorogenic **peptide** as a novel **substrate** for protein tyrosine **phosphatases**.
AU Nishikata M; Suzuki K; Yoshimura Y; Deyama Y; Matsumoto A
CS Department of Dental Pharmacology, Hokkaido University School of Dentistry, Sapporo 060-0813, Japan.. mnishika@den.hokudai.ac.jp
SO The Biochemical journal, **(1999 Oct 15)** Vol. 343 Pt 2, pp. 385-91.
AB Mca-Gly-Asp-Ala-Glu-Tyr(PO(3)H(2))-Ala- Ala-Lys(DNP)-Arg-NH(2), where Mca is (7-methoxycoumarin-4-yl)acetyl and DNP is 2,4-dinitrophenyl, was synthesized as a fluorogenic **substrate** for protein tyrosine **phosphatases**

(PTPs). In the **peptide**, the **fluorescent** Mca group is **quenched** efficiently by the DNP group. Although the **fluorescence** intensity of the **substrate** was practically unchanged upon PTP-catalysed dephosphorylation, it increased approx. 120-fold upon subsequent treatment with chymotrypsin. Analysis by HPLC showed that chymotrypsin cleaved only the dephosphorylated **substrate** at the Tyr-Ala bond. Thus with the aid of chymotrypsin, dephosphorylation of the **substrate** can be measured **fluorometrically**. A strictly linear correlation was observed between PTP concentration and dephosphorylation rate. The fluorogenic **substrate** was dephosphorylated by some PTPs much more rapidly than the corresponding (32)P-labelled **substrate** used for comparison, whereas alkaline **phosphatase** dephosphorylated the two **substrates** at similar rates. The fluorogenic **substrate** is therefore more specific for PTPs than the radiolabelled **substrate**. The assay with the fluorogenic **substrate** could be applied to the estimation of kinetc parameters and measurement of PTP activity in crude-enzyme preparations. The lower detection limit of our assay (1 microM **substrate** in 200 microliter of reaction mixture) was estimated to be 0.2-0.4 pmol, whereas it was estimated to be about 1 pmol in the assay that used (32)P-labelled **peptide** (specific radioactivity of approx. 1000 c.p.m. /pmol). Our assay is simple, specific, highly sensitive and non-radioisotopic, and hence would contribute greatly to the development of PTP biology.

L23 ANSWER 109 OF 369 CA COPYRIGHT 2007 ACS on STN
AN 128:151089 CA
TI **Assays** for protein **kinases** using **fluorescent** protein **substrates**
IN Tsien, Roger Y.; Cubitt, Andrew B.
PA Regents of the University of California, USA; Tsien, Roger Y.; Cubitt, Andrew B.
SO PCT Int. Appl., 69 pp.

PI	**WO 9802571**	**A1**	**19980122**	**WO 1997-US12410**	**19970716**
	US 5912137	**A**	**19990615**	**US 1996-679865**	**19960716**
PRAI	US 1996-679865	A1	19960716		

AB This invention provides **assays** for protein **kinase** activity using **fluorescent** proteins engineered to include sequences that can be **phosphorylated** by protein **kinases**. The proteins exhibit different **fluorescent** properties in the non-**phosphorylated** and **phosphorylated** states. Aequorea victoria green **fluorescent** proteins contg. substitution mutations were prepd. with recombinant Escherichia coli. Some displayed increased **fluorescence** upon **phosphorylation**, other decreased **fluorescence**.

L23 ANSWER 269 OF 369 CA COPYRIGHT 2007 ACS on STN
AN 117:247747 CA
TI **Measurement** of enzyme activity with labelled **peptide substrates**
IN Ikenaka, Tokuji; Mega, Tomohiro; Hamazume, Yasuki
PA Wako Pure Chemical Industries, Ltd., Japan
SO U.S., 12 pp. Cont.-in-part of U.S. Ser. No. 32,253, abandoned.

PI	**US 5120644**	**A**	**19920609**	**US 1988-256078**	**19881006**
PRAI	JP 1986-76349	A	19860401		

AB Enzymes that modify proteins are **assayed** using **peptide substrates** with a **fluorescent** label. Enzymes that are **assayed** include endopeptidases,

proteinases, **transferases**, **kinases**, and **phosphatases**. The synthesis of the renin **substrate** 2-pyridyl-Pro-Phe-His-Leu-Val-Tyr-β-Ala from 2-pyridyl glycine and BOC amino acids is described. This was used as an **assay substrate** for renin with the **fluorescent** cleavage product clearly separable from the **substrate** by HPLC.

```
=> log y
STN INTERNATIONAL LOGOFF AT 21:26:49 ON 24 JUL 2007

=> d his

     (FILE 'HOME' ENTERED AT 19:04:38 ON 24 JUL 2007)
     FILE 'CA' ENTERED AT 19:04:50 ON 24 JUL 2007
L1    1 S DICKENS ?/AU AND BIOCHEM?/SO AND BIOPHYS?/SO AND 1991/PY
L2    0 S ROSSOMONDO ?/AU AND 1992/PY
L3    1 S ROSSOMONDO ?/AU
L4   10 S MITOGEN AND KINASE AND 1992
L5  181 S MITOGEN AND KINASE AND 1992/PY
L6   16 S L5 AND SCI?/SO
L7    9 S L6 AND NAT?/SO
L8   13 S PEARSON ?/AU AND BIOL?/SO AND 1985/PY
L9    2 S L8 AND CHEM?/SO
L10   2 S L7 AND ROSSOMANDO ?/AU
L11   5 S L1,L9-10

=> d bib,ab,it 1-5

L11  ANSWER 2 OF 5  CA  COPYRIGHT 2007 ACS on STN
AN   117:85711  CA
TI   The phorbol ester-dependent activator of the mitogen-activated protein
     kinase p42mapk is a kinase with specificity for the threonine and
     tyrosine regulatory sites
AU   Rossomando, Anthony; Wu, Jie; Weber, Michael J.; Sturgill, Thomas W.
CS   Dep. Intern. Med., Univ. Virginia, Charlottesville, VA, 22908, USA
SO   Proceedings of the National Academy of Sciences of the United States of
     America (1992), 89(12), 5221-5
AB   Mitogen-activated protein kinases (MAP kinases) are activated by dual
     tyrosine and threonine phosphorylations in response to various stimuli,
     including phorbol esters. To define the mechanism of activation,
     recombinant wild-type 42-kDa MAP kinase (p42mapk) and a kinase-defective
     mutant of p42mapk (K52R) were used to assay both activator activity for
     p42mapk and kinase activity toward K52R in stimulated EL4.IL2 mouse
     thymoma cells. Phorbol 12,13-dibutyrate (10 min, 650 nM) stimulated a
     single peak of MAP kinase activator that was coeluted from Mono Q at pH
     7.5 and 8.9 with K52R kinase activity. Both activities were inactivated
     by the serine/threonine-specific phosphoprotein phosphatase 2A but not
     by the tyrosine-specific phosphoprotein phosphatase CD45.
     Phosphorylation of K52R occurred specifically on Thr-183 and Tyr-185, as
     detd. by tryptic phosphopeptide mapping in comparison with synthetic
     marker phosphopeptides. These findings indicated that phorbol ester-
     stimulated MAP kinase kinase can activate p42mapk by threonine and
     tyrosine phosphorylations, and that p42mapk thus does not require an
     autophosphorylation reaction.
```

L11 ANSWER 3 OF 5 CA COPYRIGHT 2007 ACS on STN
AN 114:224317 CA
TI Phosphorylation of tyrosines 1158, 1162 and 1163 on a synthetic dodecapeptide by the insulin receptor protein-tyrosine kinase
AU **Dickens, Martin**; Tavare, Jeremy M.; Clack, Beatrice; Ellis, Leland; Denton, Richard M.
CS Sch. Med. Sci., Univ. Bristol, Bristol, BS8 1TD, UK
SO **Biochemical and Biophysical Research Communications** (**1991**), 174(2), 772-8
AB To investigate the mechanism of tyrosine phosphorylation by insulin receptor kinase, a synthetic dodecapeptide substrate (RRDIYETDYYRK; amino acids 1155-1165) contg. the 3 major insulin receptor autophosphorylation sites was utilized. All 3 tyrosines on this peptide were rapidly phosphorylated, and phosphorylation was probably initiated at Tyr-9. This peptide thus serves as a useful tool to study the mechanism of transphosphorylation by the insulin receptor. A proteolytic activity was detected in purified receptor prepns. that removed basic residues from the peptide and prevented its binding to phosphocellulose paper. Such activity could pose a serious problem when using peptide substrates to assay for protein kinases in other acellular systems.

L11 ANSWER 4 OF 5 CA COPYRIGHT 2007 ACS on STN
AN 103:209706 CA
TI Substrate specificity of a multifunctional calmodulin-dependent protein kinase
AU **Pearson, Richard B.**; Woodgett, James R.; Cohen, Philip; Kemp, Bruce E.
CS Repatriation Gen. Hosp., Univ. Melbourne, Heidelberg, 3081, Australia
SO **Journal of Biological Chemistry** (**1985**), 260(27), 14471-6
AB The substrate specificity of the multifunctional calmodulin-dependent protein kinase from skeletal muscle was studied by using a series of synthetic peptide analogs. The enzyme phosphorylated a synthetic peptide corresponding to the N-terminal 10 residues of glycogen synthase (Pro-Leu-Ser-Arg-Thr-Leu-Ser-Val-Ser-Ser-NH2), stoichiometrically at serine (Ser)-7, the same residue phosphorylated in the parent protein. The synthetic peptide was phosphorylated with a Vmax of 12.5 μmol/min/mg and an apparent Km of 7.5 μM compared to values of 1.2 μmol/min/mg and 3.1 μM, resp., for glycogen synthase. Similarly, a synthetic peptide corresponding to the N-terminal 23 residues of smooth muscle myosin light chain was readily phosphorylated on Ser-19 with a Km of 4 μM and a Vmax of 5.4 μmol/min/mg. The importance of the arginine found 3 residues N-terminal to the phosphorylated serine in each of these peptides was evident from expts. in which this arginine was substituted by either leucine or alanine, as well as from expts. in which its position in the myosin light chain sequence was varied. Positioning arginine-16 at residues 14 or 17 abolished phosphorylation, whereas location at residue 15 not only decreased Vmax 14-fold but switched the major site of phosphorylation from Ser-19 to threonine-18. The sequence Arg-X-Y-Ser(Thr) (where X and Y are any amino acids) apparently represents the min. specificity determinant for the multifunctional calmodulin-dependent protein kinases. Studies with various synthetic peptide substrates and their analogs revealed that the specificity determinants of the multifunctional calmodulin-dependent protein kinase

were distinct from several other arginine-requiring protein kinases.

L11 ANSWER 5 OF 5 CA COPYRIGHT 2007 ACS on STN
AN 103:33969 CA
TI Spatial requirements for location of basic residues in peptide substrates for smooth muscle myosin light chain kinase
AU Kemp, Bruce E.; **Pearson, Richard B.**
CS Dep. Med., Univ. Melbourne, Heidelberg, 3081, Australia
SO **Journal of Biological Chemistry** (**1985**), 260(6), 3355-9
AB The requirement of basic residues as substrate specificity determinants for chicken gizzard myosin light-chain kinase (I) was studied by using synthetic peptide analogs of the local phosphorylation site sequence in the myosin light chains, Lys-Lys-Arg13-Pro-Gln-Arg16-Ala-Thr-Ser19-Asn-Val-Phe-Ala. The basic residue arginine (Arg)-16, exerted a strong influence on the kinetics of phosphorylation similar to that reported previously for the 3-adjacent residues, lysine (Lys)-11, Lys-12, and Lys-13. The location of Arg-16 in relation to serine (Ser)-19 as well as the distance between Arg-13 and Arg-16 had a profound effect on both the kinetics and the site specificity of phosphorylation. Placement of Arg-16 at position 15 resulted in a complete switch in phosphorylation site specificity from Ser-19 to threonine (Thr)-18. Increasing the no. of alanine residues between Arg-13 and Arg-16 in the model peptide, Lys-Lys-Arg-(Ala)n-Arg-Ala-Thr-Ser-Asn-Val-Phe-Ala, also influenced the kinetics and site specificity of peptide phosphorylation. With 2 or 3 alanines (n = 2 or 3), the apparent Km was 7.5 and 10 μM, resp., and 97% of the phosphate was esterified to Ser-19. Increasing or decreasing the no. of alanines (n = 0 to n = 4) was accompanied by an increase in the apparent Km and phosphorylation of both Thr-18 and Ser-19. These results supported the concept that both the presence and location of basic residues play an essential role in the substrate specificity of the smooth muscle I.

=> log y
STN INTERNATIONAL LOGOFF AT 19:24:50 ON 24 JUL 2007